12. (Amended) The polynucleotide of claim 11, wherein the mutation is within 50 nucleotides of an [ISDR] interferon sensitivity determining region (ISDR) or includes the ISDR.

70. (Amended) A host cell comprising the polynucleotide of claim [2] 1, wherein the host cell is a mammalian cell.

72. (Amended) The host cell of claim [71] 70 wherein the host cell is a human cell.

REMARKS

Claims 1, 3-24, 29, 41-44, 61-70 and 72-75 are pending in this application.

Election/Restriction

The Patent Office has made final the Restriction requirement of Paper No. 5. Applicants assert that the Restriction is improper. The claims as currently pending all depend from claim 1. Thus, all of the claims of this application are directed to the same invention, i.e., polynucleotides that comprise an adaptive mutation that confers improved cell culture characteristics to the polynucleotide. Thus, if claim 1 of this application is found to be patentable, all of the claims currently pending would necessarily be patentable. The polynucleotides of Group II do not have a separate and distinct structure from the polynucleotides of Group I, as all of the claims in both Group I and Group II are drawn to polynucleotides that comprise an adaptive mutation. The Group II claims merely add an additional element, specifically the IRES. Those claims are directed to the same invention as claim 1, as those claims must comprise the adaptive mutation of claim 1 because they are dependent thereon. Thus, no separate and additional search is required, and therefore examination of all of the pending claims would not constitute an undue burden.

Applicants maintain the position that the restriction is improper, and respectfully request reconsideration and withdrawal of the restriction requirement.

Priority

The Patent Office has determined that the priority date of the claims of the instant application is the same as the filing date of the instant application, May 23, 2000.

Applicants respectfully point out that the claims of the instant application are supported by the specification of the parent application (09/034,756) as follows.

The specification of that patent application, at page 14, lines 15-18 discusses the adaptation of infectious HCV nucleic acids for propagation in vitro. At page 16, line 27 through page 17, line 1 of that specification the method by which identification of such adaptive mutations can be achieved is described in detail. Specifically, incorporation of a dominant selectable marker into the infectious HCV would allow one to select for HCV variants with adaptive mutations that allow for increased levels of replication of HCV in a cell line in culture. This methodology is further discussed at page 41, lines 10-25, where the concept of engineering a dominant selectable marker under the control of the HCV replication machinery is discussed. This method would allow for efficient selection of adaptive variants capable of higher levels of replication *in vitro*. Then, at pages 95-96 of the 09/034,756 specification, an example is presented wherein a genetic construct comprising a dominant selectable marker, NEO, is inserted into an infectious DNA clone of HCV, which was transfected into a human hepatocyte cell line and then was selected for neomycin resistance. In this way, HCV with higher levels of replication can be selected for, thus allowing for the identification of adaptive HCV variants. Such variants can be sequenced by methods known in the art in order to identify the particular mutations that allow for more efficient replication of HCV *in vitro*.

Thus, the claims of the current application are in fact supported by the parent specification. Applicants have pointed out specifically where in the specification of the parent application support for the adaptive mutations can be found, as requested by the Patent Office. In view of the above discussion, applicants respectfully request that the claims of the present application be afforded a priority date of March 4, 1997, the filing date of provisional application number 60/039,843, to which the parent application, 09/034,756 claims priority.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1, 3-9, 12-17, 29, 61-62 and 69-75 are rejected under 35 U.S.C. §112, second paragraph as being indefinite. Specifically, the Office asserts that the specification only discloses mutations of the NS5A gene, and accordingly the metes and

bounds of the claimed invention cannot be determined. The Office also asserts that since the adaptive mutations can encompass mutations that render the polynucleotide capable of replication in a non-hepatic cell or which cause the polynucleotide to have attenuated virulence, the scope of the claims is unclear.

The specification, at page 61, line 24 through page 62, line 3 describes in addition to several mutations in the NS5A gene, mutations in the NS3 and NS4B genes. The claims are directed to HCV polynucleotides that comprise adaptive mutations and the specification provides examples of such mutations in several locations of the HCV coding region, specifically in the NS5A, NS3 and NS4B genes. It is expected that such mutations can be identified in other locations also. Such mutations are sufficiently representative of the genus of mutations that allow for enhanced HCV replication efficiency in vitro that the claims as now pending are not indefinite. Thus, the mutations identified in the specification represent a genus of such mutations that exist across many locations in the HCV genome, and accordingly the claims are directed to polynucleotides that comprise adaptive mutations located anywhere in the claimed polynucleotide. As such, the claims are not indefinite.

With respect to the characteristics of the adaptive mutations, the claims have been amended to recite adaptive mutations that confer improved cell culture characteristics. Improved cell culture characteristics are discussed for example at page 26, line 9 through page 27, line 5. In addition, the Example provides further clarification as to cell culture characteristics and provides methods of evaluating particular mutations for the ability to confer such characteristics to the claimed HCV polynuceotide. Thus, the claims are not indefinite with respect to the characteristics of the adaptive mutations.

Claims 70 and 72 have been amended to correct dependency, as suggested in the Office Action.

In view of the amendments to the claims and the remarks above, applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

Rejections under 35 U.S.C. §112, first paragraph

Claims 1-9, 29, 61-62 and 69-75 are rejected under 35 U.S.C. §112, first paragraph because the specification is not enabling for polynucleotides comprising adaptive mutations other than of the NS5A gene. The factors considered by the Office in rejecting the claims will be addressed in turn below.

The nature of the invention: The Office correctly identifies the invention as encompassing polynucleotides comprising among other things any and all adaptive mutations which render the polynucleotide capable of replication in a non-hepatic cell. The invention is not directed to mutations that cause the polynucleotide to have attenuated virulence, as indicated by the amendment to claim 1.

The state of the prior art and the predictability or lack thereof in the art: The Office relies on Yanagi et al., U.S. Patent No. 6,153,421 as representative of the state of the art relative to the predictability of adaptive mutations that do not render the polynucleotide incapable of productive replication in a host cell. The Office interprets that reference as teaching adaptive mutations as described and claimed in the instant application. However, the Yanagi patent fails to teach mutations that confer improved cell culture characteristics. The description is focused on an infectious DNA clone of HCV. The examples describe the construction of several clones, and further describes how these clones were transfected into the livers of chimpanzees in order to assess the infectivity of the clones. Nowhere in that specification are any particular mutations described, nor are the effects of any such mutations evaluated. The cited portion of the specification merely speculates that preferred genes in which mutations could be located include, *but are not limited to,* the P7, NS4B and NS5A genes. The authors merely speculate as to the preferred genes for mutation; and in fact no teaching as to the phenotype that any such mutation would confer is put forth. The Office states in the Action that this reference teaches that adaptive mutations are limited to deletion of part or all of the polynucleotide portions encoding the P7, NS4B and NS5A genes. That statement is incorrect. The Yanagi reference and the Office's interpretation of that reference does not correctly represent the state of the art at the time. As mentioned above, the cited portion of Yanagi et al. merely puts forth a guess as to the most likely places that a mutation would be found. There are multiple phenotypic characteristics

mentioned that could potentially be achieved by such mutations, including for example attenuated virulence. The Yanagi reference merely confirms that at the time the instant application was filed, no adaptive mutations had in fact been identified in HCV. The state of the art at the time teaches that adaptive mutations that confer improved cell culture characteristics can be identified. Such mutations had at the time been identified for many other viruses, including for example Hantavirus, Sindbis virus and HAV. See for example: Lundkvist et al., *Cell culture adaptation of Puumala Hantavirus changes the infectivity for its natural reservoir,* Clethrionomys glareolus, *and leads to accumulation of mutants with altered genomic RNA S segment.* J. Virol. 71:9515-9523 (1997); Frolov et al., *Selection of RNA replicons capable of noncytopathic replication in mammalian cells.* J. Virol. 73:3854-3865 (1999); Jansen et al., *Complete nucleotide sequence of a cell culture-adapted variant of hepatitis A virus: comparison with wild-type virus with restricted capacity for in vitro replication.* Virology 163:299-307 (1988). Thus, at the time the instant application was filed, the state of the art taught that there is an expectation that adaptive mutations could be identified in other viruses, such as HCV. Until a replicating HCV clone was made possible by the instant inventors' discovery of the 3' NTR which is necessary for HCV replication, it was not possible to identify adaptive mutations that confer improved cell culture characteristics. Once this hurdle was overcome, the inventors of the instant application were successful in identifying such mutations, as expected in view of the state of the art.

The amount of direction or guidance present and the presence or absence of working examples: The Office incorrectly states that the specification only discloses how to make adaptive mutations in the NS5A gene. As discussed above, the specification provides methods that can be used to make and identify any and all adaptive mutations. The Example describes methods for assembly of a selectable replicon and transcription and transfection of the RNA transcripts into cells. The Example also describes the establishment of G418-resistant cell clones and the identification of adaptive mutations in the NS5A, NS3 and NS4B genes that confer improved cell culture characteristics. Thus, the amount of guidance in the specification is sufficient to teach how to make adaptive mutations, as evidenced by the fact that the described method was utilized to identify several actual examples of adaptive

mutations. The Example in the specification is an actual working example of a method for identifying adaptive mutations and actual examples of such mutations in the NS5A, NS3 and NS4B genes, which provides sufficient guidance for a skilled artisan to identify any and all adaptive mutations without undue experimentation. As pointed out by the Office, undue experimentation is interpreted to mean experimentation that requires 'ingenuity beyond that to be expected of one of ordinary skill in the art' Fields v. Conover, 170 USPQ 276 (CCPA 1971) or requiring an extended period of experimentation in the absence of sufficient direction or guidance, In re Colianni, 195 USPQ 150 (CCPA 1977). As discussed above, the specification provides sufficient guidance in the form of an actual working example of a method to select for and identify adaptive mutations, such that a skilled artisan could make and use polynucleotides that comprise adaptive mutations that confer improved cell culture characteristics without undue experimentation.

Further, other researchers have since used essentially the same methods described in the instant application to identify mutations that confer improved cell culture characteristics, confirming that no undue experimentation is necessary to practice the claimed invention. Krieger et al. (J. Virol. 75:4614-4624, 2001) and Lohmann et al. (J. Virol. 75:1437-1449, 2001) describe the use of essentially the same methods taught in the instant application, to successfully identify adaptive mutations in HCV. Adaptive mutations were identified in all of the NS proteins, except for NS4A. Thus, those references provide further evidence that the claims as currently pending are enabled such that the skilled artisan can make and use the invention without undue experimentation.

Based on the amendments to the claims and the remarks above, applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph for lack of enablement.

Rejections under 35 U.S.C. §102

Claims 1, 7, 9-11, 61-62, 69-70, 72 and 73-74 are rejected under 35 U.S.C. §102(e) as being anticipated by Yanagi et al. Specifically, the Office alleges that Yanagi et al. teach an embodiment of the polypeptides in which part or all of the portion of the

polynucleotide encoding the NS5A protein is deleted, and that deletions in the polynucleotides encoding the infectious nucleic acid sequences may be made in order to produce attenuated HCV for vaccine development. Applicants contend that at best, Yanagi et al. make a suggestion to try to produce such mutated HCV polynucleotides, because they did not show any adaptive mutants or teach any method for producing such mutants, or provide any guidance as to what phenotypic characteristics that any particular mutations would confer. In addition, the Office cites Yanagi as teaching attenuating mutations. The instant claims are directed to HCV polynucleotides with mutations that confer improved cell culture characteristics, and therefore Yanagi does not anticipate the instant claims.

Yanagi teaches an infectious DNA clone of HCV. A careful reading of that reference reveals that the only mention of mutations within the HCV polynucleotide are those cited in the Office Action. Those portions, critically, fail to mention any specific mutations, any methods for inducing such mutations, nor any means for predicting exactly where such mutations should be made. There is also no teaching of any means of predicting exactly what affect, if any, a particular mutation will have on the HCV polynucleotide. Since Yanagi et al. fail to teach any actual mutations, the reference fails to teach each and every element of the instant claims. Therefore, rejection of the claims under 35 U.S.C. §102(e) is improper.

Rejections under 35 U.S.C. §103

Claims 3-6 and 29 are rejected under 35 U.S.C. §102(e) as anticipated, or in the alternative, under 35 U.S.C. §103(a) as obvious over Yanagi et al.

As discussed above, Yanagi fails to teach each and every element of the claims. Specifically, Yanagi et al. fails to teach any mutation (let alone a mutation that confers improved cell culture characteristics), and therefore Yanagi et al. cannot, alone, properly be used as a basis for rejecting the claims either under 35 U.S.C. §102 or 35 U.S.C. §103. A prima facie case of anticipation or obviousness requires that each and every element of a claim be taught by the reference(s) cited. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Yanagi merely mentions that mutations are possible, and may result in a variety of phenotypes. No guidance is given with respect to how a

skilled artisan would go about identifying a mutation that confers a particular phenotypic characteristic. Without such guidance, Yanagi does not provide an enabling disclosure, nor does that reference teach *any* mutations or the phenotypic characteristics that such a mutation confers, and as such cannot properly be considered to anticipate or render obvious the instant claims. Applicants respectfully request that this rejection be withdrawn.

Claims 12-13 and 15-16 are rejected under 35 U.S.C. §102(e) as anticipated by, or in the alternative under 35 U.S.C. §103(a) as obvious over Yanagi et al. in light of Gale et al. (Virology 230:217-227, 1997).

As above, the instant claims require a mutation that confers improved cell culture characteristics, an element that is not taught by either the Yanagi reference or the Gale reference. Gale merely teaches that the ISDR is located within the NS5A gene. Thus, that reference does nothing to overcome the fact that Yanagi et al. do not teach any mutations that confer improved cell culture characteristics, as discussed above. Therefore, the rejection is improper. Applicants respectfully request withdrawal of this rejection.

Rejections under 35 U.S.C. §103

Claims 8 and 75 are rejected under 35 U.S.C. §103(a) as being unpatentable over Yanagi et al in view of Mizuno et al. (Gastroenterology 109:1933-40, 1995).

Yanagi is cited as teaching an HCV polynucleotide comprising a sequence that is capable of productive replication in a host cell. Mizuno is cited as teaching that HeLa cells can be transfected with HCV polynucleotides. As discussed above, the references cited fail to teach each and every element of the instant claims. Yanagi provides no teaching of mutations of any kind, and no teaching of adaptive mutations, an element required by the instant claims. Mizuno et al. is not cited as teaching, and in fact does not teach, any adaptive mutations. Therefore, the combination of references cited fails to meet the requirements for *prima facie* obviousness. Applicants respectfully request reconsideration and withdrawal of this rejection.

Double Patenting

Claims 69-73 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting over claims 95-97 of copending Application No. 09/917,563. It is unclear, based on the text of the Office Action at section 12, whether this rejection is intended to be a statutory or non-statutory double patenting rejection, as the form paragraphs are directed to statutory double patenting and the language of the rejection itself is directed to a non-statutory rejection. Applicants do not believe that a rejection under either theory is proper in this instance. Applicants respectfully point out that the cited claims are not the same as the instant claims, in that the instant claims 69-73 are directed to cells comprising polynucleotides comprising an adaptive mutation that confers improved cell culture characteristics. The claims of the '563 application do not require an adaptive mutation. The '563 claims, therefore do not recite each and every limitation of the instant claims, and therefore are not coextensive in scope, and thus a rejection under statutory double patenting is improper. Further, the Action alleges that the claims of the instant application and those of the '563 application are both directed to hepatocytes comprising polynucleotides comprising among other things, an adaptive mutation as the basis for the provisional obviousness-type double patenting rejection. As discussed directly above, the claims of the '563 application do not in fact require the adaptive mutation that is required by the instant claims. Therefore, the two sets of claims are patentably distinct and as such the provisional rejection is improper. Applicants respectfully request reconsideration and withdrawal of this provisional rejection.

Conclusion

It is believed that this paper is fully responsive to the Office Action of January 10, 2002. Applicants believe that the claims as currently pending are in condition for allowance and respectfully request such action.

Respectfully submitted,

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AMENDED CLAIMS

1. (Three times Amended) A polynucleotide comprising a non-naturally occurring HCV sequence that is capable of productive replication in a host cell, or is capable of being transcribed into a non-naturally occurring HCV sequence that is capable of productive replication in a host cell, wherein the HCV sequence comprises from 5' to 3' on the positive-sense nucleic acid, a functional 5' non-translated region (5' NTR); one or more protein coding regions, including at least on polyprotein coding region that is capable of replicating HCV RNA; and a functional HCV 3' non-translated region (3' NTR), wherein said polynucleotide further comprises an adaptive mutation that confers improved cell culture characteristics to said polynucleotide.

12. (Amended) The polynucleotide of claim 11, wherein the mutation is within 50 nucleotides of an [ISDR] interferon sensitivity determining region (ISDR) or includes the ISDR.

70. (Amended) A host cell comprising the polynucleotide of claim [2] 1, wherein the host cell is a mammalian cell.

72. (Amended) The host cell of claim [71] 70 wherein the host cell is a human cell.